

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1-115. **(Cancelled)**

116. **(Previously presented)** A system for control of gene expression comprising:

(i) a first nucleic acid molecule comprising a cis-repressive sequence element upstream of an open reading frame (ORF), or including part of the open reading frame, wherein the first nucleic acid molecule forms a stem-loop structure that represses translation of the ORF; and

(ii) a second nucleic acid molecule comprising first and second stem-forming portions and a non-stem-forming portion, wherein the non-stem-forming portion connects the 3' end of the first stem-forming portion and the 5' end of the second stem-forming portion to form a loop, and wherein a portion of the second nucleic acid molecule is complementary or substantially complementary to a portion of the first nucleic acid molecule and interacts with the first nucleic acid molecule to derepress translation of the ORF.

117-176. **(Cancelled)**

177. **(Previously presented)** A kit for allowing a user to regulate expression of a gene of choice comprising:

(a) a first plasmid comprising

(i) a template for transcription of a cis-repressive RNA element; and

(ii) a promoter located upstream of the template for transcription of the cis-repressive RNA element;

- (b) a second plasmid comprising
- (i) a template for transcription of a cognate trans-activating RNA element; and
 - (ii) a promoter located upstream of the template for transcription of the trans-activating RNA element; and
 - (c) one or more elements selected from the list consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.
178. **(Previously presented)** A kit for allowing a user to regulate expression of a gene of choice comprising:
- a plasmid comprising a template for transcription of a cis-repressive RNA element and a promoter located upstream of the template for transcription of the cis-repressive RNA element and further comprising a template for transcription of a cognate trans-activating RNA element and a promoter located upstream of the template for transcription of the cognate trans-activating RNA element; and
 - one or more elements selected from the list consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.
179. **(Previously presented)** A kit for allowing a user to regulate expression of a gene of choice comprising:
- (a) a first plasmid comprising
 - (i) a template for transcription of a cis-repressive RNA element; and

- (ii) a promoter located upstream of the template for transcription of the cis-repressive RNA element;
- (b) a second plasmid comprising
- (i) a template for transcription of a cognate trans-activating RNA element; and
- (ii) a promoter located upstream of the template for transcription of the trans-activating RNA element;
- (c) a third plasmid comprising a template for transcription of a cis-repressive RNA element and a promoter located upstream of the template for transcription of the cis-repressive RNA element and further comprising a template for transcription of a cognate trans-activating RNA element and a promoter located upstream of the template for transcription of the cognate trans-activating RNA element; and
- (d) one or more elements selected from the list consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.
180. **(Previously presented)** A kit comprising:
- one or more oligonucleotides comprising a crRNA sequence, one or more oligonucleotides comprising a taRNA sequence, or one or more oligonucleotides comprising a crRNA sequence and one or more oligonucleotides comprising a taRNA sequence, wherein the kit further comprises one or more items selected from the group consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

181. **(Withdrawn)** A method of regulating translation of an open reading frame comprising steps of:
- introducing an engineered template for transcription of an mRNA into a cell and allowing mRNA transcription to occur resulting in a transcribed mRNA, wherein the template is engineered so that the transcribed mRNA comprises first and second nucleic acid elements that form a stem-loop structure that represses translation of the mRNA; and
- providing an engineered nucleic acid molecule that interacts with the mRNA so as to derepress translation of the mRNA to the cell.
182. **(Withdrawn)** The method of claim 181, wherein the engineered template comprises:
- (i) a first stem-forming portion;
- (ii) a second stem-forming portion, wherein the two stem-forming portions are complementary or substantially complementary;
- (iii) a non-stem-forming portion connecting the 3' end of the first stem-forming portion and the 5' end of the second stem-forming portion; and
- (iv) an open reading frame (ORF),
- wherein the engineered nucleic acid molecule forms a stem-loop structure that represses translation of the ORF.
- 183-242. **(Canceled)**
243. **(New)** The method of claim 181, wherein the engineered nucleic acid molecule comprises:
- (i) a first stem-forming portion;
- (ii) a second stem-forming portion; and
- (iii) a non-stem-forming portion , wherein the non-stem-forming portion connects the 3' end of the first stem forming portion and the 5' end of the second stem-forming portion to form a loop,
- and wherein a portion of the nucleic acid molecule is complementary or substantially complementary, to a portion of the transcribed mRNA.

244. (New) The system of claim 116, wherein the first nucleic acid molecule represses translation by at least 80%.
245. (New) The system of claim 116, wherein the first nucleic acid molecule represses translation by at least 90%.
246. (New) The system of claim 116, wherein the first nucleic acid molecule represses translation by at least 98%.
247. (New) The system of claim 116, wherein the second nucleic acid molecule activates translation by at least 5 fold.
248. (New) The system of claim 116, wherein the second nucleic acid molecule activates translation by at least 10 fold.
249. (New) The system of claim 116, wherein the second nucleic acid molecule activates translation by at least 19 fold.
250. (New) The system of claim 116, wherein the first and second nucleic acid molecules are composed of RNA.
251. (New) The system of claim 116, wherein the first and second nucleic acid molecules are composed of DNA.
252. (New) The system of claim 116, wherein the first and second nucleic acid molecules are composed of DNA and RNA.
253. (New) The system of claim 116, wherein the nucleic acid molecule is positioned upstream of the ORF.
254. (New) The system of claim 116, wherein the first nucleic acid molecule comprises:
 - (i) a first stem-forming portion;

- (ii) a second stem-forming portion, wherein the two stem-forming portions are complementary or substantially complementary, and
- (iii) a non-stem-forming portion that forms a loop connecting the 3' end of the first stem-forming portion and the 5' end of the second stem-forming portion, wherein the engineered nucleic acid molecule forms a stem-loop structure that represses translation when positioned upstream of an open reading frame (ORF).
255. (**New**) The system of claim 254, wherein the first and second stem-forming portions are substantially complementary.
256. (**New**) The system of claim 116, wherein at least a portion of the first nucleic acid molecule is complementary or substantially complementary to a ribosome binding site (RBS).
257. (**New**) The system of claim 116, wherein at least a portion of the first nucleic acid molecule is complementary or substantially complementary to a Kozak consensus sequence.
258. (**New**) The system of claim 254, wherein the sequence of the second stem-forming portion comprises an RBS.
259. (**New**) The system of claim 254, wherein the sequence of the non-stem-forming portion comprises YUNR.
260. (**New**) The system of claim 254, wherein the non-stem forming portion is 4, 5, 6, 7, 8, 9, 10, 11, or 12 nucleotides in length.
261. (**New**) The system of claim 254, wherein the non-stem forming portion is between 13 and 50 nucleotides in length, inclusive.
262. (**New**) The system of claim 116, whereby the length of the stem is between 4 and 100 nucleotides, inclusive.

263. (New) The system of claim 116, wherein the length of the stem is between 6 and 50 nucleotides, inclusive.
264. (New) The system of claim 116, wherein the length of the stem is between 12 and 30 nucleotides, inclusive.
265. (New) The system of claim 116, wherein the length of the stem is approximately 19 nucleotides .
266. (New) The system of claim 116, wherein the stem exhibits at least 66% complementarity.
267. (New) The system of claim 116, wherein the stem exhibits between 75 and 95% complementarity.
268. (New) The system of claim 116, wherein the stem exhibits approximately 85% complementarity.
269. (New) The system of claim 116, wherein the stem includes at least one area of non-complementarity.
270. (New) The system of claim 269, wherein the stem includes at least one bulge.
271. (New) The system of claim 116, wherein the stem includes at least two dispersed areas of non-complementarity.
272. (New) The system of claim 271, wherein the stem includes at least two dispersed bulges.
273. (New) The system of claim 116, wherein the stem includes at least three dispersed areas of non-complementarity.
274. (New) The system of claim 273, wherein the stem includes at least three dispersed bulges.
275. (New) The system of claim 116, wherein the nucleic acid molecule forms a single stable stem.

276. (New) The system of claim 116, wherein the nucleic acid molecule represses translation in the absence of a ligand.
277. (New) The system of claim 116, wherein the first stem-forming portion comprises a sequence complementary or substantially complementary to a sequence in the 5' portion of an ORF.
278. (New) The system of claim 254, wherein the nucleic acid molecule comprises a start codon.
279. (New) The system of claim 278, wherein the nucleic acid molecule comprises a spacer comprising one or more nucleotides between the 3' end of the second stem-forming portion and the start codon.
280. (New) The system of claim 278, wherein all or part of the start codon is located within the second stem-forming portion.
281. (New) The system of claim 116, wherein the nucleic acid molecule comprises one or more nucleotides at the 5' end that do not participate in the stem-loop structure.
282. (New) The system of claim 116, wherein the nucleic acid molecule comprises between 5 and 50 nucleotides upstream of the 5' end of the first stem-forming portion.
283. (New) The system of claim 116, wherein the nucleic acid molecule comprises a ligand binding domain.
284. (New) The system of claim 254, wherein the nucleic acid molecule comprises a third stem-forming portion that is complementary or substantially complementary to the second stem-forming portion, wherein the first and third stem-forming portions form alternate stem-loop structures with the second stem-forming portion.
285. (New) The system of claim 284, wherein the first and third stem-forming portions comprise a portion that is complementary or substantially complementary to an RBS.

286. (New) The system of claim 116, wherein the second nucleic acid molecule comprises a portion comprising the sequence YNAR positioned 5' to the 5' portion of the first stem-forming sequence.
287. (New) The system of claim 116, wherein the length of the stem formed by the two stem-forming portions of the second nucleic acid molecule is between 6 and 50 nucleotides.
288. (New) The system of claim 116, wherein the length of the stem formed by the two stem-forming portions of the second nucleic acid molecule is between 12 and 30 nucleotides.
289. (New) The system of claim 116, wherein the length of the stem formed by the two stem-forming portions of the second nucleic acid molecule is approximately 19 nucleotides.
290. (New) The system of claim 116, wherein the two stem-forming portions of the second nucleic acid molecule exhibit at least 66% complementarity.
291. (New) The system of claim 116, wherein the two stem-forming portions of the second nucleic acid molecule exhibit between 75 and 95% complementarity.
292. (New) The system of claim 116, wherein the two stem-forming portions of the second nucleic acid molecule exhibit approximately 85% complementarity.
293. (New) The system of claim 116, wherein the stern formed by the two stem-forming portions of the second nucleic acid molecule includes at least one area of non-complementarity.
294. (New) The system of claim 116, wherein the stem formed by the two stem-forming portions of the second nucleic acid molecule includes at least two dispersed areas of non-complementarity.
295. (New) The system of claim 116, wherein the stem formed by the two stem-forming portions of the second nucleic acid molecule includes at least three dispersed areas of non-complementarity.

296. (New) The system of claim 116, wherein the second nucleic acid molecule comprises a nucleotide analog.
297. (New) The system of claim 116, wherein the second nucleic acid molecule comprises a ligand binding domain.
298. (New) The system of claim 116, wherein the first and second nucleic acid molecules interact so as to disrupt the stem-loop structure formed by the first nucleic acid molecule, thereby allowing a ribosome to gain access to a ribosome binding site.
299. (New) The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR10 and the second nucleic acid molecule has the sequence of taR10.
300. (New) The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR12 and the second nucleic acid molecule has the sequence of taR12.
301. (New) The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR10 or a variant of crR10 that differs from crR10 by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity and the second nucleic acid molecule has the sequence of taR10 or a variant of taR10 that differs from taR10 by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity.
302. (New) The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR12 or a variant of crR12 that differs from crR12 by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity and the second nucleic acid molecule has the sequence of taR12 or a variant of taR12 that differs from taR12 by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity.
303. (New) The system of claim 116, wherein the first nucleic acid molecule and the second nucleic acid molecule have an equilibrium association constant between 0.8×10^7 and 1.5×10^7 kcal/mol.